High Production Volume (HPV) Challenge Program

Test Plan

For

PHTHALIC ACID TETRABROMO BIS 2-ETHYLHEXYL ESTER (CAS# 26040-51-7)

Prepared for:

Brominated Phthalate Ester Panel (BR PEP)
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Date

July 1, 2004

TABLE OF CONTENTS

1.0 INTRODUCTION: PHTHALIC ACID TETRABROMO BIS 2-ETHYLHEXYL ESTER

The Brominated Phthalate Ester Panel (BR PEP) has voluntarily committed to participate in the High Production Volume Chemical (HPV) Challenge Program. The Brominated Phthalate Ester Panel (comprised of Teknor Apex Company, Great Lakes Chemical Corporation and Unitex Chemical Corporation) is sponsoring phthalic acid tetrabromo bis 2-ethylhexyl ester (CAS#26040-51-7) for the HPV Challenge Program.

The objective of this test plan is to evaluate the available data and determine what additional data, if any, are needed to complete the agreed screening level data set for phthalic acid tetrabromo ester. This document includes an evaluation of relevant available data. Robust summaries of these data are appended. Based upon a thorough evaluation of all existing data for phthalic acid tetrabromo ester no additional testing is proposed.

2.0 EVALUATION OF EXISTING DATA FOR PHTHALIC ACID TETRABROMO ESTER:

The available data for phthalic acid tetrabromo bis 2-ethylhexyl ester have been evaluated in accordance with the guidance developed by EPA and have been prepared as robust summaries. Most of the data were generated using chemically pure phthalic acid tetrabromo ester (e.g. >95%). Robust summaries of these files are appended.

PHYSICAL and CHEMICAL PROPERTIES:

Melting Point

The melting point has been determined to be 229.19 degrees C (mean or weighted MP; derived from MPBPWIN v1.40).

Acceptable scientific information is available; no additional testing is proposed.

Boiling Point

The boiling point has been determined to be 539.75 degrees C (adapted Stein & Brown method; derived from MPBPWIN v1.40).

Acceptable scientific information is available; no additional testing is proposed.

Vapor Pressure

The vapor pressure is 1.71E-011 mmHg @ 25 degrees C (modified Grain method; derived from MPBPWIN v.1.40).

Acceptable scientific information is available; no additional testing is proposed.

Partition Coefficient

The octanol/water partition coefficient is 11.95 Log Kow (KOWWIN v1.66 estimate).

Acceptable scientific information is available; no additional testing is proposed.

Water Solubility

The water solubility is 1.983E-009 mg/L @ 25 degrees C (WSKOW v1.40; estimated from LogKow).

Acceptable scientific information is available; no additional testing is proposed.

Photodegradation

Photodegradation occurs through the absorbance of solar radiation at levels high enough for the chemical to undergo a transformation. According to program guidance, photodegradation can be estimated using models accepted by EPA. The estimation method includes the calculation of atmospheric oxidation potential (AOP). The computer program AOPWIN (atmospheric oxidation program for Microsoft Windows) is used by EPA. It calculates the chemical half life based on an overall OH reaction rate constant, a 12 hour day, and a given OH concentration. There is no OH radical in the phthalic acid molecule. Nevertheless the AOPWIN was performed on phthalic acid tetrabromo ester. The estimate for atmospheric oxidation at 25 degrees C is listed below under "Transport and Distribution". It predicts that the half-life in air is about 0.5 days. The fugacity model gives the t ½ in air 11.8 hours, in water 1440 hours, in soil 1440 hours and in sediment 5760 hours.

The AOPWIN model, an acceptable model used by EPA, provides an estimate of the atmospheric oxidation for phthalic acid tetrabromo ester. No additional testing is proposed.

Stability in Water

The half-life at 25 degrees C and pH 7 is 29.219 days (HYDROWIN v1.67).

Acceptable scientific information is available; no additional testing is proposed.

ENVIRONMENTAL FATE and PATHWAYS:

Biodegradation

Biodegradation is the utilization of a chemical by microorganisms as a source of energy and carbon. The biodegradation of phthalic acid tetrabromo ester has been estimated using the closed bottle and modified Sturm test methods. The ThOD is 1.34 mg O_2/mg test substance. Oxygen consumption was only 0.06 and 0.05 mg O_2/mg, or 4% of its ThOD at 2 and 10 mg/L after 10 days. These values indicate the test material is not readily degradable. The COD (chemical oxygen demand) was 0.92 mgO_2/mg test substance, or 69% of its ThOD. This also demonstrates that it was not completely oxidized. CO2 production after 28 days was 1.1 and 2.3 mg for 10 and 20 mg/L test samples, respectively. This is only 2% of the TCO2, further demonstrating that the test material is not readily degradable. The test material was not sufficiently soluble to allow the calculation of a reliable estimate of degradation, such as the DOC (dissolved organic carbon).

Acceptable scientific information is available; no additional testing is proposed.

Transport/Distribution

Models for predicting bioconcentration and soil sorption have been developed and used for chemicals where water solubility is known. Although the test material is not appreciably soluble in water several bioconcentration and biodegradation estimates were determined using available models. The results are summarized here:

Henrys Law Constant (25 deg C) [HENRYWIN v3.10]
Bond Method: 2.98E-007 atm-m3/mole
Group Method: 3.08E-007 atm-m3/mole

Henrys LC [VP/WSol estimate using EPI values]: 8.012E-003 atm-m3/mole

Probability of Rapid Biodegradation [BIOWIN v4.00]
Linear Model : 0.5352
Non-Linear Model : 0.1319

Expert Survey Biodegradation Results:
Ultimate Survey Model: 1.9718 (months)
Primary Survey Model: 3.2110 (weeks)

Readily Biodegradable Probability [MITI Model]
Linear Model : 0.3604
Non-Linear Model : 0.0581

Atmospheric Oxidation (25 deg C) [AOPWIN v1.90]
Hydroxyl Radicals Reaction:
Overall OH Rate Constant = 21.8176E-12 cm3/molecule-sec
Half-Life = 0.490 days (12 hr day; 1.5E6 OH/cm3)
Half-Life = 5.883 hrs
Ozone Reaction: No estimate

Soil Adsorption Coefficient [PCKOCWIN v1.66]
Koc : 1.26E+006
Log Koc: 6.101

Aqueous Base/Acid-Catalyzed Hydrolysis (25 deg C) [HYDROWIN v1.67]
Total Kb for pH > 8 at 25 deg C : 2.74E+000 L/mole-sec
Kb Half-Life at pH 8 : 2.922 days
Kb Half-Life at pH 7 : 29.219 days

BCF Estimate from LogKow [BCFWIN v2.14]
Log BCF = 0.500 (BCF = 3.162)
Log Kow used: 11.95 (estimated)

Volatilization from Water:
Henry LC: 3.08E-007 atm-m3/mole (Group SAR Method)
Half-Life from Model River: 5054 hours (210.6 days)
Half-Life from Model Lake: 5.536E+004 hours (2307 days)

Level III Fugacity Model:

	Mass Amount (%)	Half-Life (hr)	Emissions (kg/hr)
Air	0.123	11.8	1000
Water	2.27	1.44E+003	1000
Soil	32.2	1.44E+003	1000
Sediment	65.4	5.76E+003	0

Persistence Time: 2.74E+003 hr

Acceptable scientific information is available; no additional testing is proposed.

ECOTOXICITY:

Aquatic Toxicity

Aquatic toxicity testing is required to determine the concentration of a chemical that will produce mortality or growth inhibition in 50% of a specified population (LC50 and EC50, respectively). There was only one

aquatic fish toxicity test conducted, using rainbow trout. It was a 96-hour static test. However, the test results are not considered reliable because the test material was not soluble at the concentrations used (e.g. 62.5 – 1000 mg/L). In a 48 hour Daphnia magna mortality/immobility test the EC50 was 0.30 mg/L for immobility and 0.27 mg/L for floating plus immobile daphnia. Aquatic effects were observed in the Daphnia assay only because concentrations were kept at or below 1 mg/L, the test material's limit of water solubility. An acute toxicity study to aquatic plants is not considered useful data based upon the very poor water solubility at concentrations greater 1 mg/L.

An ECOSAR analysis was also conducted and the following were estimated [ECOSAR v0.99]:

ECOSAR Class	Organism	Distribution	End Pt.	Predicted mg/L (ppm)
Neutral Organic SAR (baseline toxicity)	Fish	14 day	LC50	2.04E-006*
Esters	Fish	96 hr	LC50	0.000508*
Esters	Daphnid	48 hr	LC50	4.95E-007*
Esters	Green Algae	96 hr	EC50	5.83E-005*
Esters	Green Algae		ChV	5.55E-005*
Esters	Fish		ChV	2.37E-007*

Note: * indicates the chemical may not be soluble enough to measure this predicted effect. Fish and daphnid acute toxicity log Kow cutoff is 5.0; Green Algae EC50 toxicity log Kow cutoff is 6.4; Chronic toxicity log Kow cutoff is 8.0; MW cutoff is 1000.

Ecological effects studies that have been conducted demonstrate that the test material is not readily soluble in water. Based upon the water solubility and the results of the ECOSAR analysis that was performed, no additional testing is proposed.

HUMAN HEALTH EFFECTS:

Acute Toxicity

Oral Toxicity: The acute oral toxicity to rats was examined in a limit dose study and the LD50 was determined to be greater than 5000 mg/kg. In an acute dermal toxicity study in rabbits, the LD50 was greater than 2 ml/kg (equivalent to 3.09 g/kg).

Acceptable scientific information is available; no additional testing is proposed.

Eye and Skin Irritation

The test material produced only very slight eye and skin irritation in rabbits.

Acceptable scientific information is available; no additional testing is proposed.

Dermal Sensitization

The potential dermal sensitization was examined in guinea pigs and it was determined that the phthalic acid tetrabromo ester was not a dermal sensitizer.

Acceptable scientific information is available; no additional testing is proposed.

Repeated Dose Testing

Phthalic acid tetrabromo ester was administered in the diet to rats for 4 weeks. The dose levels were 200, 2000 and 20000 ppm. Cageside observations, clinical chemistry, hematology and urinalysis evaluations were performed. There was no evidence of systemic toxicity or mortality. Only slight body weight decrease was observed in high dose females, along with decreased calcium and phosphorous levels. A full compliment of male and female reproductive organs and tissues were examined via gross necropsy as well as histopatholgical evaluations. Organ weight evaluations (absolute and relative) of several organs also included an examination of testes and ovaries. No adverse effects on the reproductive organs or any other tissues examined were found. The NOEL was 2,000 ppm based upon the body weight decrease in females at 20,000 ppm, which is considered the NOAEL. The effects observed at 20,000 ppm are indicative of exceeding prescribed dietary limits (i.e. 10%) of active ingredient that are recognized in standardized national and international protocols. The result is reduced food intake and the associated changes are not considered compound related.

Acceptable scientific information is available; no additional testing is proposed.

Mutagenicity Assays

Gene Mutation: Phthalic acid tetrabromo ester was not mutagenic in an Ames assay in *Salmonella typhimurium.*

Chromosomal Aberration:

In Vitro: Phthalic acid tetrabromo ester was weakly positive for producing chromosome aberrations in human lymphocytes with and without metabolic activation.

In Vivo: In a mouse micronucleus assay, Phthalic acid tetrabromo ester was negative for clastogenic effects.

Acceptable scientific information is available; no additional testing is proposed for gene mutation or chromosomal aberration.

Reproductive Toxicity Studies

Effects on reproductive organs in male and female rats were examined in a 4 week repeat dose toxicity study. A full compliment of male and female reproductive organs and tissues were examined via gross necropsy as well as histopatholgical evaluations. Organ weight evaluations (absolute and relative) of several organs included an examination of testes and ovaries. No adverse effects on the reproductive organs examined were found. See the results discussed above under Repeated Dose Testing, and in the robust summary.

Acceptable evaluation of reproductive organs was performed in both sexes, at extremely high dose levels. This evaluation included blood chemistry, clinical chemistry, urinalysis, organ weights (absolute and relative), gross and histological examinations. No additional reproductive testing is proposed since an acceptable repeat dose study, covering specific endpoints, is available.

Developmental Toxicity Studies

The panel did not locate relevant, valid, developmental toxicity studies for phthalic acid tetrabromo ester. Phthalic acid tetrabromo ester is not acutely toxic, produces only slight irritation to eyes and skin, did not cause any adverse effects following 28 days of oral ingestion at extremely high dietary levels, and was not mutagenic in a battery of in vitro gene mutation and in vivo chromosomal aberrations assays. The weak

clastogenic effect in the in vitro human lymphocyte study was not observed in the in vivo mouse micronucleus assay. Additionally, a developmental toxicity study conducted in accordance with contemporary OECD Test Guidelines, using phthalic acide tetrabromo ester, would have to incorporate dose levels equal to or higher than the dose levels used in the repeated dose test. The dose levels incorporated in the repeated dose study, 2000 and 20,000 ppm, produced only slight body weight decrease in only one sex. Such dose levels are much greater than potential, and unlikely, estimated human exposures. Further, such dietary dose levels (i.e. >10% in the diet) are so high they interfere with normal dietary intake of food and other nutrients. Based upon the absence of any adverse effects in these studies, using very high dose levels, a developmental toxicity study is unlikely to provide any additional useful information.

Based upon the rationale discussed above, giving specific consideration to dose levels required, a developmental toxicity study would not provide useful scientific information that is not already available from existing data.

3.0 CONCLUSIONS

Environmental fate data demonstrate that phthalic acid tetrabromo ester is not water soluble and not readily biodegradable. Very low vapor pressure estimates and low Henry's Law constant indicate very little, if any, volatilization from water to the atmosphere.

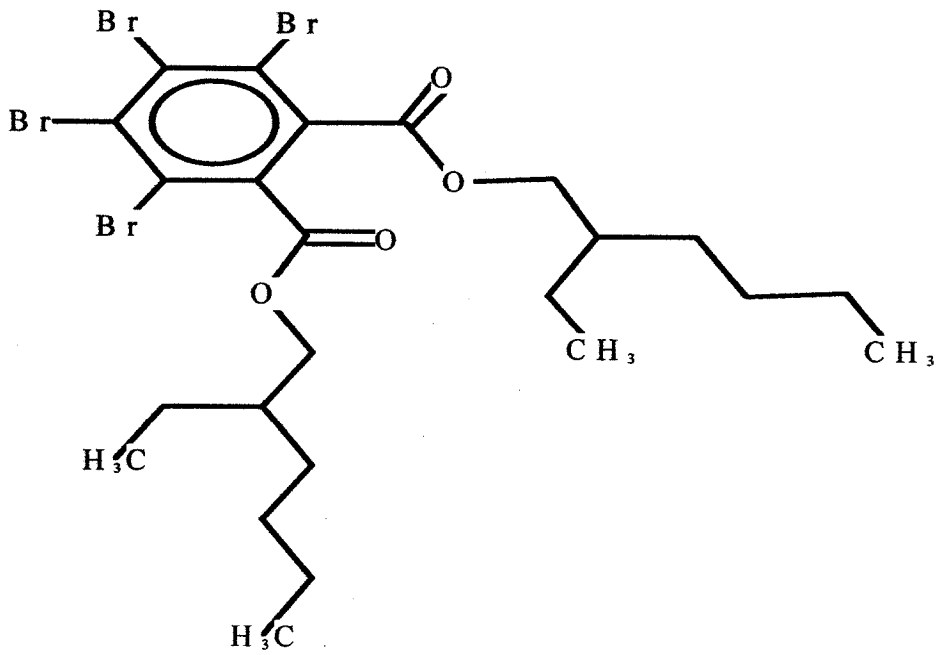
No additional environmental fate or ecological effects tests will be conducted, based upon scientifically reliable data in Daphnia magna and the demonstration that phthalic acid tetrabromo ester is not water soluble at levels >1mg/L. This was illustrated in the acute fish toxicity study in rainbow trout. Therefore, an aquatic fish toxicity study should not be repeated and an aquatic plant toxicity study would not provide useful data. An ECOSAR analysis confirms this conclusion.

There is a sufficient and reliable battery of human health effects studies. These include acute, irritation, sensitization, repeated dose, reproductive and mutagenicity test methods. There was no developmental toxicity study for phthalic acid tetrabromo ester, however, this test method is not deemed toxicologically relevant in view of the lack of any toxicity at limit dose levels in acute studies, no adverse effects on reproductive parameters at maximum dietary levels in a 28 day study, and the absence of mutagenic effects in an Ames gene mutation assay and an in vivo chromosomal aberration test. Collectively these data support the finding that phthalic acid tetrabromo ester does not produce any adverse effects in the bioassays performed at very high limit dose levels.

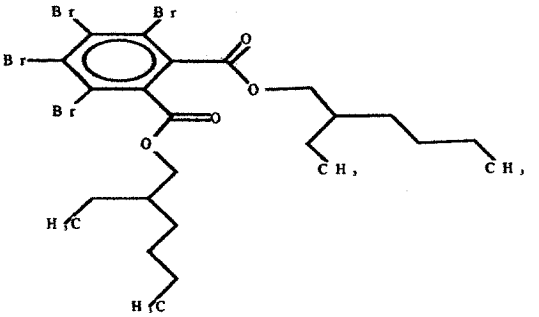
TABLE 1: HPV SIDS DATA REQUIREMENTS/CRITICAL STUDIES: Phthalic Acid Tetrabromo Ester (CAS #26040-51-7)

HPV Data Category	Test Endpoint		Data Available	Data Acceptable	Data to be Generated
Physical and Chemical Properties	Melting Point		Yes[1]	Yes	No
	Boiling Point		Yes[1]	Yes	No
	Vapor Pressure		Yes[1]	Yes	No
	Partition Coefficient		Yes[1]	Yes	No
	Water Solubility		Yes[2]	Yes	No
Environmental Fate and Pathways	Photodegradation		No		No
	Stability in Water		Yes[2]	Yes	No
	Biodegradation		Yes	Yes	No
	Transport/Distribution		Yes[1]	Yes	No
Ecotoxicity	Acute toxicity to fish		Yes[3]	Waiver	No
	Acute toxicity to aquatic invertebrates		Yes	Yes	No
	Toxicity to Aquatic Plants		No[4]	Waiver	No
	Chronic aquatic invertebrate test		NR[5]	NR	No
	Terrestrial toxicity		NR[5]	NR	No
Human Health Effects	Acute toxicity		Yes	Yes	No
	Repeated Dose		Yes	Yes	No
	Genetic Toxicity	Gene Mut.	Yes	Yes	No
		Chrom. Ab	Yes	Yes	No
	Reproductive Toxicity		Yes[6]	Yes	No
	Developmental Toxicity		No[7]	Waiver	No

Footnote 1: Estimated using EPIWIN and MPBPWIN models.
Footnote 2: See Test Plan for rationale. Use Ecotoxicity Study results.
Footnote 3: See Test Plan for rationale. A waiver is proposed for the acute toxicity to fish.
Footnote 4: See Test Plan for rationale. A waiver is proposed for the aquatic plant toxicity study.
Footnote 5: These SIDS data requirements are "conditional" and are not required for the Phthalic acid tetrabromo ester.
Footnote 6: See Test Plan for rationale. Use Repeated Dose Study results.
Footnote 7: See Test Plan for rationale. A waiver is proposed for the developmental toxicity study.



Phthalic acid, tetrabromo-, bis(2-ethylhexyl) ester



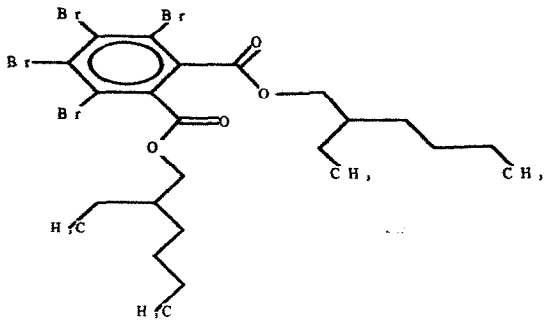
SMILES : O=C(OCC(CCCC)CC)c(c(c(c(c1Br)Br)Br)C(=O)OCC(CCCC)CC)c1Br
CHEM : Phthalic acid, tetrabromo-, bis(2-ethylhexyl) ester
CAS Num: 026040-51-7
ChemID1:
ChemID2:
ChemID3:
MOL FOR: C24 H34 Br4 O4
MOL WT : 706.15
Log Kow: 11.95 (KowWin estimate)
Melt Pt:
Wat Sol: 2.228E-007 mg/L (calculated)

ECOSAR v0.99g Class(es) Found

Esters

ECOSAR Class	Organism	Duration	End Pt	Predicted mg/L (ppm)
Neutral Organic SAR (Baseline Toxicity)	: Fish	14-day	LC50	2.04e-006 *
Esters	: Fish	96-hr	LC50	0.000508 *
Esters	: Daphnid	48-hr	LC50	4.95e-007 *
Esters	: Green Algae	96-hr	EC50	5.83e-005 *
Esters	: Green Algae		ChV	5.55e-005 *
Esters	: Fish		ChV	2.37e-007 *

Note: * = asterick designates: Chemical may not be soluble enough to measure this predicted effect.
Fish and daphnid acute toxicity log Kow cutoff: 5.0
Green algal EC50 toxicity log Kow cutoff: 6.4
Chronic toxicity log Kow cutoff: 8.0
MW cutoff: 1000



SMILES : O=C(OCC(CCCC)CC)c(c(c(c(c1Br)Br)Br)C(=O)OCC(CCCC)CC)c1Br
CHEM : Phthalic acid, tetrabromo-, bis(2-ethylhexyl) ester
CAS NUM: 026040-51-7
MOL FOR: C24 H34 Br4 O4
MOL WT : 706.15

---------- EPI SUMMARY (v3.10) ----------

Physical Property Inputs:
Water Solubility (mg/L): ------
Vapor Pressure (mm Hg) : ------
Henry LC (atm-m3/mole) : ------
Log Kow (octanol-water): ------
Boiling Point (deg C) : ------
Melting Point (deg C) : ------

Log Octanol-Water Partition Coef (SRC):
Log Kow (KOWWIN v1.66 estimate) = 11.95

Boiling Pt, Melting Pt, Vapor Pressure Estimations (MPBPWIN v1.40):
Boiling Pt (deg C): 539.75 (Adapted Stein & Brown method)
Melting Pt (deg C): 229.19 (Mean or Weighted MP)
VP(mm Hg,25 deg C): 1.71E-011 (Modified Grain method)

Water Solubility Estimate from Log Kow (WSKOW v1.40):
Water Solubility at 25 deg C (mg/L): 1.983e-009
log Kow used: 11.95 (estimated)
no-melting pt equation used

ECOSAR Class Program (ECOSAR v0.99g):
Class(es) found:
Esters

Henrys Law Constant (25 deg C) [HENRYWIN v3.10]:
Bond Method : 2.98E-007 atm-m3/mole
Group Method: 3.08E-007 atm-m3/mole
Henrys LC [VP/WSol estimate using EPI values]: 8.012E-003 atm-m3/mole

Probability of Rapid Biodegradation (BIOWIN v4.00):
Linear Model : 0.5352
Non-Linear Model : 0.1319
Expert Survey Biodegradation Results:
Ultimate Survey Model: 1.9718 (months)
Primary Survey Model : 3.2110 (weeks)
Readily Biodegradable Probability (MITI Model):
Linear Model : 0.3604
Non-Linear Model : 0.0581

Atmospheric Oxidation (25 deg C) [AopWin v1.90]:
Hydroxyl Radicals Reaction:
OVERALL OH Rate Constant = 21.8176 E-12 cm3/molecule-sec
Half-Life = 0.490 Days (12-hr day; 1.5E6 OH/cm3)
Half-Life = 5.883 Hrs
Ozone Reaction:
No Ozone Reaction Estimation

Soil Adsorption Coefficient (PCKOCWIN v1.66):
     Koc   : 1.262E+006
     Log Koc: 6.101

Aqueous Base/Acid-Catalyzed Hydrolysis (25 deg C) [HYDROWIN v1.67]:
 Total Kb for pH > 8 at 25 deg C : 2.745E+000 L/mol-sec
 Kb Half-Life at pH 8:   2.922 days
 Kb Half-Life at pH 7:   29.219 days

BCF Estimate from Log Kow (BCFWIN v2.14):
   Log BCF = 0.500 (BCF = 3.162)
      log Kow used: 11.95 (estimated)

Volatilization from Water:
   Henry LC: 3.08E-007 atm-m3/mole (estimated by Group SAR Method)
   Half-Life from Model River: 5054 hours   (210.6 days)
   Half-Life from Model Lake : 5.536E+004 hours   (2307 days)
Level III Fugacity Model:

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	0.123	11.8	1000
Water	2.27	1.44e+003	1000
Soil	32.2	1.44e+003	1000
Sediment	65.4	5.76e+003	0

    Persistence Time: 2.74e+003 hr

TABLE 1: DATA MATRIX : HUMAN HEALTH EFFECTS STUDIES FOR:

Phthalic Acid Tetrabromo Ester (CAS #26040-51-7)

CAS #	ACUTE LD50/LC50			IRRITATION		DERMAL SENSITIZ	REPEATED DOSE STUDIES	REPRO	DEVEL	GENETIC TOXICOLOGY	
	Oral	Dermal	Inhalation	Eye	Skin					Gene Mutation	Chromosome Aberration
26040-51-7	AO-1 (1)	AD-1 (1)		EI-1 (1)	DI-1 (1)	DS-1 (1)	RD-1 (1)	RD-1 (1)		MU-1 (1)	MU-2 (1)
											MU-3 (1)

Each entry denotes a study reference number, for example: AO-1, EI-1, MU-2, etc., followed by the Klimisch Rating in ( )

Klimisch Ratings are ranked as follows: (1) = reliable without restriction; (2) = reliable with restriction; (3) = not reliable; and (4) = not assignable.

Health & Environmental Horizons, Ltd. (May 14, 2003)

TABLE 2 – DATA MATRIX: ENVIRONMENTAL FATE AND ECOTOXICITY TESTS FOR:

Phthalic Acid Tetrabromo Ester (CAS# 26040-51-7)

CAS #	ENVIRONMENTAL FATE TESTS				ECOTOXICITY TESTS		
	Photodeg OECD 113	Stab.InWater OECD 111	Biodeg. OECD 301 302, 308	Tran/Dist. EQC MOD	Aquatic OECD 203	Invert. OECD 202	Plants OECD 201
26040-51-7		EPIWIN	BD-1 (1)	EPIWIN	AF-1 (3)	AINV-1 (2)	

[Health & Environmental Horizons, Ltd. (October 16, 2003)]

TABLE 3 – DATA MATRIX: CHEMICAL and PHYSICAL PROPERTY TESTS FOR:

Phthalic Acid Tetrabromo Ester (CAS # 26040-51-7)

CAS #	CHEMICAL and PHYSICAL PROPERTIES				
	Melting Point	Boiling Point	Vapor Pressure	Partition Coefficient	Water Solubility
26040-51-7	**1**	**1**	**1**	**2**	**3 & 4**

Footnote **1**: Estimated using MPBPWIN v1.40
Footnote **2**: Estimated using KOWWIN v1.66
Footnote **3**: Estimated using WSKOW v1.40
Footnote **4**: See Acute Toxicity to Fish, AF-1 and Acute Toxicity to Aquatic Invertebrates, AINV-1

[Health & Environmental Horizons, Ltd., (October 16, 2003)]

A. ACUTE TOXICITY:

1. REPORT NUMBER: AO-1

STUDY TYPE: Acute Oral Toxicity to Rats

TEST MATERIAL: Slightly viscous; light yellow liquid; FR-45B, RC9927, CAS No. 26040-51-7, Code No. 6159-199-3, Sp. gravity 1.545 @ 25°C/4°C; purity >95%.

TESTING FACILITY: Life Science Research, Ltd

STUDY NUMBER(S): 86/PTC013/634

SPONSOR: Pennwalt Corporation

REPORT ISSUED or STUDY COMPLETION DATE: March 11, 1987

TITLE OF REPORT: Acute Oral Toxicity Test in the Rat;

AUTHOR: Joseph F. Jadlocki and Joel A. Seckar, Ph.D.

RECOGNIZED METHOD, i.e. OECD: OECD Guideline 401 and EPA Toxic Substances Control Act Test Guidelines (1985)

GLP: Yes.

SPECIES/SEX: Charles River CD Rats; males and female; young adults (5 weeks old); males weighing 117 grams and females weighing 116 grams.

DOSE LEVEL(S) and NUMBER OF DOSES: 1 dose of 5000 mg/kg suspended in maize (corn) oil; a 50% v/v solution.

NUMBER OF ANIMALS/DOSE: 5 Male/5 Female

MEASURED ENDPOINT/INDEX (i.e. LC50, symptoms): Lethality after 14 days

STUDY METHOD: Groups of 5 male and 5 female Charles River CD rats were administered a single oral dose of 5000 mg/kg of test material in a constant volume (10 ml/kg). Rats were grouped housed. Food and water available ad libitum, except for overnight period prior to administration, during which food was withheld but water was available. Test material administered via gavage (syringe). Cageside observations of animals were performed 3 times during the first hour after dosing and up to 5 hours post-dosing, after which they were observed twice daily until Day 15. Body weights were measured before testing, day 1 of testing and weekly thereafter. A complete gross necropsy was performed on each rat.

RESULTS/OBSERVATIONS: No mortality occurred at the single dose of 5000 mg/kg. Rats exhibited normal body weight gain. No toxic symptoms or signs were reported. There were no adverse internal or external effects observed in any rat at necropsy.

DATA QUALITY: Study was conducted in accordance with a recognized scientific procedure for analyzing the acute oral toxicity of a test material in experimental animals conforming to an OECD Limit Dose study. The study fully supports scientific standards and provides sufficient information to support the conclusion that the acute oral LD50 in male/female albino rats is greater than 5000 mg/kg.

RELIABILITY:

1. w/o restriction [X]
2. w restriction [ ]
3. not reliable [ ]
4. not assignable [ ]

Comments: Table 2 describes the "volume-dosage" as "10 mg/kg" and should be "10 ml/kg". Tables 1, 3 and 4 correctly report the value as ml/kg.

2. REPORT NUMBER: DI-1

STUDY TYPE: Primary dermal irritation to rabbits

TEST MATERIAL: Slightly viscous; light yellow liquid; FR-45B, RC9927, CAS No. 26040-51-7, Code No. 6159-199-3, Sp. gravity 1.545 @ 25°C/4°C; purity >95%.

TESTING FACILITY: Life Science Research, Ltd

STUDY NUMBER(S): 86/PTC015/682

SPONSOR: Pennwalt Corporation

REPORT ISSUED or STUDY COMPLETION DATE: January 21, 1987

TITLE OF REPORT: Acute Dermal Irritation/Corrosion Test in the Rabbit

RECOGNIZED METHOD, i.e. OECD: OECD Guideline 404 and EPA Toxic Substances Control Act Test Guidelines (1985)

GLP: Yes.

SPECIES/SEX: Young (3 months) albino outbred New Zealand rabbits; both sexes; weighing 2.08-3.14 kg.

DOSE LEVEL(S) and NUMBER OF DOSES: 0.5 ml of test material was impregnated onto a gauze pad and then applied to the shaved intact skin site on the back.

NUMBER OF ANIMALS/DOSE: 3 males and 3 females.

MEASURED ENDPOINT/INDEX (i.e. LD50, PII):Test material was considered to be a very slight dermal irritant. A primary dermal irritation score was not reported.

STUDY METHOD: 24 hours prior to conducting the study, the dorsum was clipped free of hair, careful not to damage the skin to be used as test sites. 0.5 ml of test material was impregnated onto a gauze pad and then applied to the shaved intact skin site on the back. These skin sites were wrapped with gauze bandages and held in place for 4 hours. After 4 hours the bandages were removed, excess test material was removed with a moistened towel and the test sites were observed for erythema and edema. Dermal responses were scored at 1, 24, 48 and 72 hours after patch removal.

RESULTS/OBSERVATIONS: Very slight erythema (score of 1) was observed in 3 rabbits within one hour after patch removal. At 24 hours only 1/3 rabbits had very slight erythema. This rabbit was negative at 48 hours. No edema was observed at any time.

DATA QUALITY: Study was conducted in accordance with a recognized scientific procedure for analyzing the primary dermal irritation of a test material in experimental animals. Study details were sufficient to support the conclusions in the report regarding the dermal irritation of the test material.

RELIABILITY:

1. w/o restriction [X]
2. w restriction [ ]
3. not reliable [ ]
4. not assignable [ ]

3. REPORT NUMBER: EI-1

STUDY TYPE: Eye irritation to rabbits

TEST MATERIAL: Slightly viscous; light yellow liquid; FR-45B, RC9927, CAS No. 26040-51-7, Code No. 6159-199-3, Sp. gravity 1.545 @ 25°C/4°C; purity >95%.

TESTING FACILITY: Life Science Research, Ltd

STUDY NUMBER(S): 86/PTC016/686

SPONSOR: Pennwalt Corporation

REPORT ISSUED or STUDY COMPLETION DATE: January 21, 1987

TITLE OF REPORT: Acute Eye Irritation/Corrosion in the Rabbit

RECOGNIZED METHOD, i.e. OECD: OECD Guideline 405 and EPA Toxic Substances Control Act Test Guidelines (1985)

GLP: Yes.

SPECIES/SEX: Young (3 months) albino outbred New Zealand rabbits; both sexes; weighing 2.74-3.54 kg.

DOSE LEVEL(S) and NUMBER OF DOSES: 0.1 ml of test material was instilled into the everted lower eye lid of one eye of each rabbit.

NUMBER OF ANIMALS/DOSE: 6 rabbits; 3 of each sex.

MEASURED ENDPOINT/INDEX (i.e. LD50, PII): Test material produced very slight injection of the conjunctival blood vessels in 6/6 rabbits at 1 hour. All scores were zero at 24 hours.

STUDY METHOD: 24 hours prior to conduct of the test, the eyes of each rabbit were examined and determined to be free of irritation and abnormalities. On the day of testing, 0.1 ml of test material was instilled into the everted lower eye lid of one eye of each rabbit. The eyes were not rinsed. Eyes were examined at 1, 24, 48, and 72 hours after washing. Corneal opacity (when present) is confirmed using fluorescein.

RESULTS/OBSERVATIONS: The scores recorded for cornea, iris and conjunctiva were provided for all rabbits at all reading times. The test material produced very slight conjunctival irritation at 1 hour in all 6 rabbits. Redness observed in all rabbits with a score of 1 in 5/6 and 2 in 1/6 rabbits; discharge observed in 4/6 rabbits with a score of 1 in 3/6 and 2 in 1/6. All scores were zero at 24 hours. A primary eye irritation score was not calculated.

DATA QUALITY: Study was conducted in accordance with a recognized scientific procedure for analyzing the primary eye irritation of a test material in experimental animals. Study details were sufficient to support the conclusions in the report regarding the eye irritation of the test material.

RELIABILITY:

1. w/o restriction [X]
2. w restriction [ ]
3. not reliable [ ]
4. not assignable [ ]

Note: Para 4.3 Pre-exposure, test report (page 4) erroneously reports the animal weight in "grams" and should be listed as "kilograms".

4. REPORT NUMBER: AD-1

STUDY TYPE: Acute dermal toxicity to rabbits

TEST MATERIAL: Slightly viscous; light yellow liquid; FR-45B, RC9927, CAS No. 26040-51-7, Code No. 6159-199-3, Sp. gravity 1.545 @ 25°C/4°C; purity >95%.

TESTING FACILITY: Life Science Research, Ltd

STUDY NUMBER(S): 86/PTC014/676

SPONSOR: Pennwalt Corporation

REPORT ISSUED or STUDY COMPLETION DATE: January 21, 1987

TITLE OF REPORT: Acute Percutaneous Toxicity in the Rabbit

RECOGNIZED METHOD, i.e. OECD: OECD Guideline 402 and EPA Toxic Substances Control Act Test Guidelines (1985)

GLP: Yes.

SPECIES/SEX: Young (3 months) albino outbred New Zealand rabbits; both sexes; weighing 2.4-3.14 kg.

DOSE LEVEL(S) and NUMBER OF DOSES: A single dose of 2 ml of test material per kg body weight was applied to the shaved intact skin site on the back.

NUMBER OF ANIMALS/DOSE: 5 males and 5 females.

MEASURED ENDPOINT/INDEX (i.e. LD50, PII): The LD50 was greater than 2 ml/kg (equivalent to 3.09 g/Kg).

STUDY METHOD: 24 hours prior to conducting the study, the dorsum was clipped free of hair, careful not to damage the skin to be used at test sites. A dose of 2.0 ml of test material was applied to the shaved intact skin site on the back. Based upon the specific gravity this is equivalent to a dose of 3.09 g/kg. These skin sites were wrapped with gauze bandages and held in place for 24 hours. After 24 hours the bandages were removed, excess test material was removed with a moistened towel and the test sites were observed for skin reactions. Cageside observations of animals were performed 3 times during the first hour after dosing and up to 5 hours post-dosing, after which they were observed twice daily until Day 15. Body weights were measured before testing, day 1 of testing and weekly thereafter. A complete gross necropsy was performed on each rabbit.

RESULTS/OBSERVATIONS: There was no mortality at 2 ml/kg. Occasional areas of exfoliation at the treatment site were observed in only 1/10 rabbits; no other external dermal effects were observed. Gross necropsy revealed dark thyroids, thymus, lungs and salivary glands, petechiae of the thymus and/or abnormal gastrointestinal contents in the majority of rabbits examined. The laboratory did not consider these to be adverse effects based upon historical laboratory records for rabbits.

DATA QUALITY: Study was conducted in accordance with a recognized scientific procedure for analyzing the acute dermal toxicity of a test material in experimental animals. Study details were sufficient to support the conclusions in the report regarding the acute dermal toxicity of the test material.

RELIABILITY:

1. w/o restriction [X]
2. w restriction [ ]
3. not reliable [ ]
4. not assignable [ ]

5. REPORT NUMBER: DS-1

STUDY TYPE: Skin Sensitization in Guinea Pigs

TEST MATERIAL: Slightly viscous; light yellow liquid; FR-45B, RC9927, CAS No. 26040-51-7, Code No. 6159-199-3, Sp. gravity 1.545 @ 25°C/4°C; purity >95%.

TESTING FACILITY: Life Science Research, Ltd

STUDY NUMBER(S): 87/PTC012/056

SPONSOR: Pennwalt Corporation

REPORT ISSUED or STUDY COMPLETION DATE: March 6, 1987

TITLE OF REPORT: Delayed Contact Hypersensitivity Study in Guinea-Pigs

RECOGNIZED METHOD, i.e. OECD: OECD Guideline 406 and EPA Toxic Substances Control Act Test Guidelines (1985)

GLP: Yes.

SPECIES/SEX: Dunkin-Hartley albino guinea pigs; weighing 315-484 gm; both sexes.

DOSE LEVEL(S) and NUMBER OF DOSES: 0.25 ml of undiluted test material. Animals were caged 2 per sex per cage.

NUMBER OF ANIMALS/DOSE: 20 test (10 male/10 female) and 10 control g. pigs (5 male/5 female).

MEASURED ENDPOINT/INDEX (i.e.Sensitization):Test material did not produce delayed contact hypersensitivity in guinea pigs.

STUDY METHOD: Tested in accordance with the Buehler Patch Test (1965), one of several sensitization methods acceptable to OECD. 20 animals were allocated to test groups and 10 to a vehicle control group. Prior to the induction phase, material was tested to determine the concentrations to use, and the highest non-irritating concentration. On the day before applications, the hair from the backs of the g. pigs was removed by clipping, allowing a space for 4 concentrations on each animal.

Induction phase: On the day following clipping, test material was applied to patches and then taped to the upper left quadrant once a week for three weeks (Day 1, 8 and 15). After an exposure period of 6 hours, the patches were removed. On the day following application each site was scored for irritation.

Challenge phase: Two weeks after the last exposure (Day 28) all test and control animals are challenged in the same manner with patches on the right upper flank. The challenge dose was 0.25 ml of undiluted test material held in contact for 6 hours. 24 hours after application of test material the test sites were depilated with cream of calcium thioglycolate and scored to give a 24 hour and 48 hour reading.

RESULTS/OBSERVATIONS: There were no dermal responses to occluded application of 10%, 30%, 50% or undiluted test material in paraffin oil. Thus, the undiluted test material was chosen for the induction phase. There was no positive skin sensitization reaction suggestive of hypersensitivity for the test material. The positive control, 0.1% dinitrochlorobenzene in ethanol, produced a grade 1 erythema in 8/9 surviving controls (1 positive control was killed in extremis and replaced).

DATA QUALITY: Study followed the protocol of Buehler, an acceptable test method for dermal sensitization, and supports the conclusion that the test material did not product contact hypersensitivity in guinea pigs.

RELIABILITY:

1 w/o restriction	[X]
2 w restriction	[ ]
3 not reliable	[ ]
4 not assignable	[ ]

6. REPORT NUMBER: RD-1

STUDY TYPE: 4-week Dietary Repeated Dose Study in Rats

TEST MATERIAL: Pale yellow liquid; RC9927; FR-45B; CAS No. 26040-51-7; Code No. 6458-68-1; Sp. gravity 1.545 @ 25°C/4°C; purity >95%.

TESTING FACILITY: Life Science Research, Ltd

STUDY NUMBER(S): 87/PSV003/926

SPONSOR: Pennwalt Corporation

REPORT ISSUED or STUDY COMPLETION DATE: April 7, 1988

TITLE OF REPORT: Toxicity Study By Dietary Administration to CD Rats for Four Weeks

RECOGNIZED METHOD, i.e. OECD: OECD Guideline 407 and EPA Toxic Substances Control Act Test Guidelines (1985)

GLP: Yes.

SPECIES/SEX: Male/female Sprague-Dawley CD Rats

AGE at Start of Test: 5-6 weeks

ROUTE: Dietary

DOSE LEVEL(S) and NUMBER OF DOSES: 0, 200, 2000 and 20,000 ppm were incorporated into the diet and administered to rats every day for 28 days. Analysis of dietary concentrations demonstrated dose level equivalents of 0, 22, 223.4, and 2331 mg/kg. A positive control group (Di-2-ethyl hexyl phthalate) was also used and incorporated into positive control diets at 15,000 ppm.

NUMBER OF ANIMALS/DOSE: 10M/10F per dose, housed 5/cage; positive control had 5/sex.

BODY WEIGHT MEASUREMENTS: Measured prior to first dose, then weekly throughout study, and at termination.

CAGESIDE OBSERVATIONS: These were performed twice daily.

FOOD CONSUMPTION/FOOD EFFICIENCY: Diet consumption measured by weighing the feeder in each cage.

HEMATOLOGY: Blood samples were collected from the retro-orbital sinus in each rat after 26 days of treatment following overnight fasting. The following were analyzed: Packed cell volume (PCV), hemoglobin (Hb), erythrocyte count (rbc), reticulocyte count, total leukocyte count (wbc), differential leukocyte count (neutrophils, lymphocytes, eosinophils, basophils, monocytes), platelet count, mean cell hemoglobin (MCH), mean cell volume (MCV), mean cell hemoglobin concentration (MCHC), and prothrombin time (PT).

CLINICAL CHEMISTRY: Blood samples used for clinical chemistry evaluations were collected at the same time and same manner as the hematological samples. The following were determined: alkaline phosphatase (AP), alanine amino transferase (ALT), aspartate amino transferase (AST), urea, creatinine, glucose, total protein, electrophoretic protein fractions, sodium, potassium, chloride, calcium and inorganic phosphorus.

URINALYSIS: Urine samples were collected from all rats in control and high dose groups after 23 days of treatment. Samples collected overnight during food and water restriction. The following were determined: appearance, volume, pH, specific gravity, protein, total reducing substances, glucose, ketones, bilirubin, urobilin, nitrite, blood. A microscopic analysis of the sediment was also performed.

STATISTICAL METHODS: The significance of differences between dosed and control group means were assessed using Student's t-test, Fisher's Exact Probability Test and Dunnett's Test.

ORGAN WEIGHTS: Absolute and relative (organ-body) organ weights were determined for adrenals, brain, heart, kidneys, liver, lungs, pituitary, spleen, thymus, thyroid, uterus and gonads (testes and ovaries).

GROSS PATHOLOGY: A full compliment of tissues were fixed and examined grossly in all animals; including a full compliment of reproductive organs in both sexes.

HISTOPATHOLOGY: A full compliment of tissues were fixed and examined microscopically in 5M/5F animals in the control and high dose groups; including a full compliment of reproductive organs in both sexes. Any macroscopically abnormal tissues from all dose groups were also examined microscopically. Electron microscopy was performed in livers of all rats to assess peroxisome proliferation.

CLINICAL OBSERVATIONS: No evidence of systemic toxicity. Survival was unaffected by dose.

FINDINGS/MEASURED ENDPOINT/INDEX (i.e. LOAEL, NOAEL): Slight body weight decrease observed in high dose females (91% of control) and a decrease in alanine amino transferase activity ($p<0.05$). Also observed in high dose females were decreased calcium and phosphorus levels. There were no other adverse effects observed for hematology, clinical chemistry, urinalysis, organ weights, gross or microscopic analyses. Electron microscopy of livers was negative for peroxisome proliferation. The

positive control, DEHP, produced marked signs of toxicity in stark contrast to negative control and test material groups. The LOAEL for the test material was 20,000 ppm, and the NOAEL was 2,000 ppm.

DATA QUALITY: Study was conducted in accordance with a recognized scientific procedure for determining the adverse effects of a test substance when administered repeatedly in the diet for 4 weeks in experimental animals. Study was conducted in compliance with GLP regulations. The study meets national and international scientific standards and provides sufficient information to support the conclusions regarding the NOAEL and the LOAEL demonstrated from the study data.

RELIABILITY:

1 w/o restriction	[X]
2 w restriction	[ ]
3 not reliable	[ ]
4 not assignable	[ ]

B. GENETIC TOXICITY

1. REPORT NUMBER: MU-1

STUDY TYPE: *Salmonella typhimurium* Plate Assay

TEST MATERIAL: Slightly turbid, viscous pale yellow liquid; FR-45B, RC9927, CAS No. 26040-51-7, Code No. 6159-199-3, Sp. gravity 1.545 @ 25°C/4°C; purity >95%.

TESTING FACILITY: Life Science Research, Ltd

STUDY NUMBER(S): 86/PTC018/601

SPONSOR: Pennwalt Corporation

REPORT ISSUED or STUDY COMPLETION DATE: January 8, 1987

TITLE OF REPORT: Assessment of Mutagenic Potential in Histidine Auxotrophs of *Salmonella Typhimurium* (The Ames Test)

RECOGNIZED METHOD, i.e. OECD: Procedure followed Ames et al. (1975), OECD test guideline 471 (1983), and EPA Toxic Substances Control Act (1985).

GLP: Yes.

TEST ORGANISM USED: *Salmonella typhimurium* strains: TA98, TA100, TA1535, TA1537 and TA1538; provided by Dr. B.N. Ames, Berkeley, CA, USA.

TEST COMPOUND CONCENTRATIONS USED: 5 concentrations were evaluated with appropriate vehicle and positive controls. Doses used: 50, 158, 500, 1580 and 5000 ug/plate administered in DMSO (0.1 ml).

CONTROL MATERIALS: The following control materials were employed.

Positive Control:
1. Non-activation:
   9-Aminoacridine (50 ug/plate): TA1537
   2-Aminoanthracene (5 ug/plate): TA1535
   sodium azide (2 ug/plate): TA1535 and TA100

benzo[a]pyrene (5 ug/plate): TA98, TA100, TA1537 and TA1538
2-nitrofluorene (5 ug/plate); TA98, TA1538

2. Metabolic Activation:
benzo[a]pyrene (5 ug/plate): TA98, TA100, TA1537 and TA1538
2-Aminoanthracene (5 ug/plate): TA1535

Negative control: DMSO

ACTIVATION: S-9 fraction derived from Aroclor 1254 induced liver of male rats; composition fully described (per ml): 0.4 mM $MgCl_2.6H_20$/1.6M KCl, G-6-P (0.1 mM); NADP (0.1 mM); potassium phosphate-sodium phosphate pH 7.4 (0.1 M) and S-9 (10%). S-9 prepared fresh just prior to use.

TEST PERFORMANCE: Standard plate assay for gene mutations in bacterial cells.

PROTOCOL:
Preliminary toxicity tests were performed to assess the dose levels for use in the mutagenicity assays. The test material was prepared in DMSO and transferred to a molten histidine-deficient top-agar, maintained at 45°C. Duplicate samples were prepared. Three serial 10-fold dilutions in top-agar were prepared from each preparation, providing 8 different concentrations of test material ranging from 2.5 ug to 5 mg per plate. Tubes were inoculated with TA98 and incubated at 37°C for 2 days. They were then examined for the presence of background lawn of non-revertant colonies. The highest level of test material chosen is the lowest level causing visible thinning in the background lawn. Since no concentration produced this effect a 5 mg/plate was selected as the top dose.

Mutagenicity assay was carried out according to Ames et al. (1975). Each dose level of the compound was tested with and without the S9 mix with each strain of *S. typhimurium* (TA98, TA100, TA1535, TA1537 and TA1538). Cells from a culture of each indicator strain were added to test tubes containing 2 ml of molten histidine-deficient agar supplemented with S9 mix, where appropriate, and maintained at 45°C. The ingredients were thoroughly mixed and immediately poured onto the minimal glucose agar plates. Aliquots of a 10^{-6} dilution of culture were spread over the surface of the plates. After the top agar had set, the plates were incubated at 37 degrees C for 2 days. Plates were scored for number of revertants/plate. All determinations were made in triplicate, including controls. Each test, in each strain, was conducted on two separate occasions.

REPORT RESULTS: Test compound did not induce a significant increase in the number of revertant colonies over that shown in the solvent control plates for any of the *Salmonella typhimurium* strains with and without S9 activation, at doses up to and including 5000 ug/plate. Positive controls produced the expected response in all experiments.

CONCLUSION: Test material was not mutagenic in *S. typhimurium* TA 98, TA100, TA1535, TA1537 or TA1538 with and without S9 activation.

DATA QUALITY: Study was conducted in accordance with a recognized published scientific procedure for examining the mutagenic potential of a test compound in *S. typhimurium* bacteria strains. Test method utilized recognized positive controls that gave the expected positive responses, confirming the sensitivity of the method. Dose levels administered were adequate for evaluating the mutagenic potential.

RELIABILITY:

1 w/o restriction	[X]
2 w restriction	[ ]
3 not reliable	[ ]
4 not assignable	[ ]

2. REPORT NUMBER: MU-2

STUDY TYPE: Micronucleus Cytogenetic Assay in Mice.

TEST MATERIAL: Light yellow liquid; FR-45B, RC9927, CAS No. 26040-51-7, Code No. 6159-199-3, Sp. gravity 1.545 @ 25°C/4°C; purity >95%.

TESTING FACILITY: Life Science Research, Ltd

STUDY NUMBER(S): 87/PSV001/300

SPONSOR: Pennwalt Corporation

REPORT ISSUED or STUDY COMPLETION DATE: July 28, 1987

TITLE OF REPORT: Assessment of Clastogenic Action on Bone Marrow Erythrocytes in the Micronucleus Test

RECOGNIZED METHOD, i.e. OECD: OECD 474; EPA Toxic Substances Control Act (1985). The assay followed the procedure of Schmid, The Micronucleus Test, Mut. Res. 31, pg 9-15 (1975).

GLP: Yes

TEST ANIMAL: CD-1 male and female mice (4-5 weeks old). Animals were housed in single-sex groups of one, two or five.

TEST COMPOUND CONCENTRATIONS USED: Test material was administered to animals via intraperitoneal and dermal routes. A preliminary toxicity test was performed using the intraperitoneal route in doses of 250, 500, 1000 and 2000 mg/kg. The volume of the dosage was 10 ml/kg. All animals were killed 72 hours after treatment. The intraperitoneal route used dose levels of 80, 400 and 2000 mg/kg. The dermal route used 2000 mg/kg administered on 5 separate occasions, 24 hours apart.

CONTROL MATERIALS: Corn oil was used as a negative control. Chlorambucil (30mg/kg), administered orally, was used as a positive control.

TEST PERFORMANCE:

Preliminary toxicity test (intraperitoneal route): Mice were administered the dose test material in a single dose of 250, 500, 1000 or 2000 mg/kg and then sacrificed after 72 hours. Animals were killed via cervical dislocation following carbon dioxide inhalation. Femurs were removed, dissected and bone marrow samples were collected. These samples were centrifuged, the pellet collected, then resuspended, air-dried and fixed in methanol. These slides were stained using the May-Gruenwald and Giemsa technique. At least 2000 erythrocytes per animal were examined. They were scored as to polychromatic or mature. At least 1000 cells of each type were scored from each animal, where possible. Based upon these results the doses were selected for the main dermal and intraperitoneal assays. Since there was no evidence of inhibition of cell division the highest dose, 2000 mg/kg, was selected as the highest dose in the main studies.

Main Study: Intraperitoneal route: Details of slide preparation: 24, 48, and 72 hrs after dose administration 5M/5/F per test article-treated and vehicle control groups are sacrificed by CO_2 asphyxiation. Positive control group sacrificed 24 hrs after dosing. Immediately after sacrifice, femurs are exposed and bone marrow aspirated into a syringe containing fetal bovine serum and transferred to a centrifuge tube where the bone marrow cells are pelleted and the supernatant drawn off. Cells resuspended by aspiration with a capillary pipette and a small drop of the bone marrow suspension is spread onto a clean glass slide, air dried, fixed by dipping in methanol, and stained with May-Gruenwald-Giemsa. In addition to erythrocytes

being scored, the presence of micronucleated cells per 1000 erythrocytes were also identified. The proportion of polychromatic erythrocytes to total erythrocytes was also determined as an indication of inhibition of cell division. The frequency of micronuclei in polychromatic cells provides an indication of genetic damage.

Main Study: Dermal Route: Male and female mice were administered 2000 mg/kg dermally on 5 separate occasions with 24 hour intervals between dosing. It was mixed with corn oil and applied to the shaven dorsal skin. Animals were killed at 18 and 48 hours after the final treatment.

REPORT RESULTS: There was no increase in the number of micronucleated erythrocytes in bone marrow of treated mice, whether administered via the intraperitoneal or dermal routes.

Dose mg/kg Intraperitoneal	Micronucleated Cells/1000 cells					
	Polychromatic			Mature		
Both Sexes	24hr	48hr	72hr	24hr	48hr	72hr
Untreated control	1.1	0.4	1.2	0.2	0.2	0.4
Chlorambucil	47			0.9		
80 mg	1.6			0.5		
400 mg	1.1			0.8		
2000 mg	1.2	1.1	0.7	0.6	0.6	0.4

Dose mg/kg Dermal	Micronucleated Cells/1000 cells					
	Polychromatic			Mature		
Both Sexes	18hr	48hr		18hr	48hr	
Untreated control	0.7	1.8		0.5	0.6	
2000 mg	1.3	1.5		0.2	0.3	

CONCLUSION: The test material was negative (not clastogenic) in the micronucleus test using male and female CD-1 mice.

DATA QUALITY: Study was conducted in accordance with a recognized procedure for determining the frequency of micronucleated erythrocytes using bone marrow samples from mice that had been exposed to repeated dermal exposures or single intraperitoneal injections of the test substance. The assay followed GLP regulations. The study meets national and international scientific standards for in vivo assay procedures for examining the occurrence of chromosomal aberrations in treated animals.

RELIABILITY:

1. w/o restriction [X]
2. w restriction [ ]
3. not reliable [ ]
4. not assignable [ ]

NOTE: The report does not clarify whether the test material was occluded or simply applied to the unwrapped skin.

3. REPORT NUMBER: MU-3

STUDY TYPE: In Vitro Chromosome Aberration Assay in Human Lymphocytes.

TEST MATERIAL: Slightly viscous, pale yellow liquid; FR-45B, RC9927, CAS No. 26040-51-7, Code No. 6159-199-3, Sp. gravity 1.545 @ 25°C/4°C; purity >95%.

TESTING FACILITY: Life Science Research, Ltd

STUDY NUMBER(S): 87/PTC017/004

SPONSOR: Pennwalt Corporation

REPORT ISSUED or STUDY COMPLETION DATE: March 18, 1987

TITLE OF REPORT: In Vitro Assessment of the Clastogenic Activity of RC9927 in Cultured Human Lymphocytes

RECOGNIZED METHOD, i.e. OECD: OECD 473; EPA Toxic Substances Control Act (1985).

GLP: Yes

TEST CULTURE: Peripheral blood samples from an adult male human were collected, added to complete culture media and phytohemagglutinin to stimulate lymphocyte division. The collected samples were incubated at 37°C for 48 hours. Samples were then centrifuged, the supernatant removed and the cell pellet resuspended in treatment medium. Some medium contained S-9 mix (freshly prepared and derived from livers of male CD rats treated with Aroclor 1254). Vehicle control (DMSO), positive control and test solution vials were incubated at 37°C for 2 hours. Cultures were centrifuged and cells washed with treatment medium. Cells were resuspended in complete culture medium and test solution, vehicle or positive control solutions were added to each vial. Each vial was then incubated at 37°C for 22 hours. Three hours before terminating the incubation period, colcemid was added to each vial to arrest cell division. At the conclusion of the incubation period vials were centrifuged, cells harvested and resuspended in hypotonic potassium chloride for 10 minutes. Once again they were centrifuged and fixed in methanol:glacial acetic acid.

TEST COMPOUND CONCENTRATIONS USED: A preliminary cytotoxicity test was performed at dose concentrations of 1.6, 8, 40, 200 and 1000 ug/ml. Based upon these results the dose concentrations for the cytogenetic assay were selected. They were 40, 200, and 1000 ug/ml.

CONTROL MATERIAL: DMSO was used as a negative control and was used as the vehicle for the test material. Chlorambucil without S-9 (1 ug/ml) and cyclophosphamide with and without S-9 (6 ug/ml) were used as positive controls.

TEST PERFORMANCE:

Preliminary toxicity test: Single drops of the prepared cell suspensions were added to clean glass slides and air-dried. Slides were made from each culture, stained with Giemsa, washed in buffer and air-dried. Approximately 1000 lymphocytes/culture were examined and the mitotic index calculated. The dose that produced a decrease in mitotic activity was selected as the highest dose concentration. Since the highest dose tested failed to decrease mitotic activity it was selected as the highest concentration for the cytogenetic assay.

Main Cytogenetic Assay: Slides prepared as above and 1000 cells were scored and the mitotic index calculated. The following morphological observations were included: gaps, breaks, fragments, exchanges, multiple aberrations, endoreduplication, pulverized metaphases, and polyploidy. Frequencies of each aberrant metaphase were measured for each culture.

REPORT RESULTS: There was no difference between activated and non-activated mitotic indices and therefore, when considered together there were 13.2, 12.2, 12.7 and 12.7 for 0, 40, 200 and 1000 ug/ml, respectively. Thus, there was no evidence of toxicity to dividing lymphocytes.
There was also no difference in the frequency of chromosomal aberrations between activated and non-activated systems. Since there was no difference the results from replicate treatments were compared from pooled samples of activated and non-activated systems. The positive control, cyclophosphamide, showed a significant increase in aberrations in S-9 solutions compared to non-S-9 solutions.

Chromosomal aberrations were scored from 100 metaphases. Comparison of vehicle control to dose concentrations demonstrated a weak clastogenic effect at 1000 ug/ml.

Dose Group Ug/ml	S-9 Mix (+/-)	Number of cells scored	Mean Mitotic Index	Cells with Aberrations			Cells with Aberrations other than gaps		
				Total	Range %	Mean %	Total	Range %	Mean %
0	+/-	600	13.2	8	1-3	1.3	3	0-1	0.5
40	+/-	600	12.2	17	2-4	2.8	5	0-2	0.8
200	+/-	600	12.7	12	0-3	2	5	0-2	0.8
1000	+/-	600	12.7	25	3-5	4.2	16	2-4	2.7
Cycloph	-	300	11.6	7	1-4	2.3	4	0-3	1.3
	+	300	9.1	13	19-29	24.3	49	10-20	16.3
Chloram	-	300	6.5	97	29-37	32.3	68	19-26	22.7

Dose Group Ug/ml	S-9 Mix (+/-)	Single Strand gaps	Double Strand gap	Single strand break	Double strand break	Fragment	Exchange	Other
0	+/-	5	0	0	0	3	0	0
40	+/-	12	0	2	0	4	0	0
200	+/-	5	2	0	1	4	0	0
1000	+/-	11	0	5	0	12	0	0
Cycloph	-	4	0	1	0	2	0	1*
	+	23	9	10	5	31	8	0
Chloram	-	35	5	17	1	51	13	0

Footnote: * is an endoreduplication

CONCLUSION: The test material was weakly clastogenic at 1000 ug/ml in cultured human lymphocytes.

DATA QUALITY: Study was conducted in accordance with a recognized procedure for determining the in vitro chromosomal aberrations in cultured human lymphocytes. The assay followed GLP regulations. The study meets national and international scientific standards for in vitro assay procedures for examining the occurrence of chromosomal aberrations in cultured cells.

RELIABILITY:

1. w/o restriction [X]
2. w restriction [ ]
3. not reliable [ ]
4. not assignable [ ]

BIODEGRADATION AND ECOLOGICAL EFFECTS STUDIES:

1. REPORT NUMBER: BD-1

STUDY TYPE: Ready biodegradability: Modified Sturm Test

TEST MATERIAL: Pale yellow clear liquid; Pyronil 45; Code 6605-57; halogenated phthalate ester; RC9927; FR-45B; purity 95%; specific gravity = 1.545 @25°C/4°C; empirical formula: $C_{24}H_{34}O_4Br_4$; CAS No. 26040-51-7.

TESTING FACILITY: Life Science Research, Ltd

STUDY NUMBER(S): 89/PSV032/0244

SPONSOR: Pennwalt Corporation

REPORT ISSUED or STUDY COMPLETION DATE: July 10, 1989

TITLE OF REPORT: Pyronil 45: Assessment of its Ready Biodegradability. Modified Sturm Test

RECOGNIZED METHOD: Study was conducted in accordance with a recognized scientific procedure for biodegradation and is based on OECD 301D: Closed bottle test and OECD 301B, Biotic degradation Modified Sturm test.

GLP: Yes.

EXPOSURE PERIOD: 28 days

INOCULUM: The inoculum was secondary effluent obtained from a trickling-filter plant at a sewage treatment works that treats predominantly domestic waste. It was maintained under aerobic conditions in the lab until required. It was then vacuum-filtered through filter paper and the filtrate used as the inoculum.

TEST PROCEDURE: The chemical oxygen demand (COD) was determined by oxidation with acid-dichromate mixture. The test material has a low aqueous solubility and therefore, 0.46-0.78 mg of test material were directly applied to glass cover slips and added to COD vials, together with 2 ml of water. Concentrations ranged from 230-390 mg/l.

Bacterial inhibition test:
Seven groups of BOD (Biological Oxygen Demand) bottles were prepared:

1 mineral salts medium
2 inoculum
3 inoculum + sodium benzoate (2 mg/L)
4 inoculum + test material (2 mg/L)
5 inoculum + test material (10 mg/L)
6 inoculum + test material (2 mg/L) + sodium benzoate (2 mg/L)
7 inoculum + test material (10 mg/L) + sodium benzoate (2 mg/L)

The test concentrations were prepared by direct addition of test material, on glass cover slips, to BOD bottles to yield nominal concentrations of 2 and 10 mg/L. The concentration of dissolved oxygen (DO), pH and temperature were measured at the start of the test, after 5 days of incubation at 20°C, and after 10 days of incubation.

Modified Sturm test:
Four vessels (3.5 L), containing mineral salts (MSMS), bacterial sludge, test material or sodium benzoate, were prepared as follows:

1 inoculated MSMS
2 inoculated MSMS + sodium benzoate (20 mg/L)
3 inoculated MSMS + test material (10 mg/L)
4 inoculated MSMS + test material (20 mg/L)

- Weights of the test material were 35 and 70 mg, added directly to the solutions. The closed bottle test was performed according to the EEC/OECD test guidelines (No. 301 D). Prior to the test, CO2 was removed by flushing with air that had been passed through Carbosorb ASI, 10N sodium hydroxide, and 0.025N barium hydroxide for 24 hours. In the closed bottle test, the test compound was added to an aqueous solution of mineral salts and exposed to relatively low numbers of microorganisms under aerobic conditions for a period of 28 days. Samples were taken throughout the study for pH, temperature and dissolved organic carbon (DOC).

- The following endpoints were determined:
  - ThOD (theroretical oxygen demand) of the test material
  - TCO_2 (theroretical carbon dioxide) generated by the test material
  - COD (chemical oxygen demand) of each sample
  - BOD (biological oxygen demand) in the bacterial inhibition test
  - CO_2 production by control, reference and test mixtures..

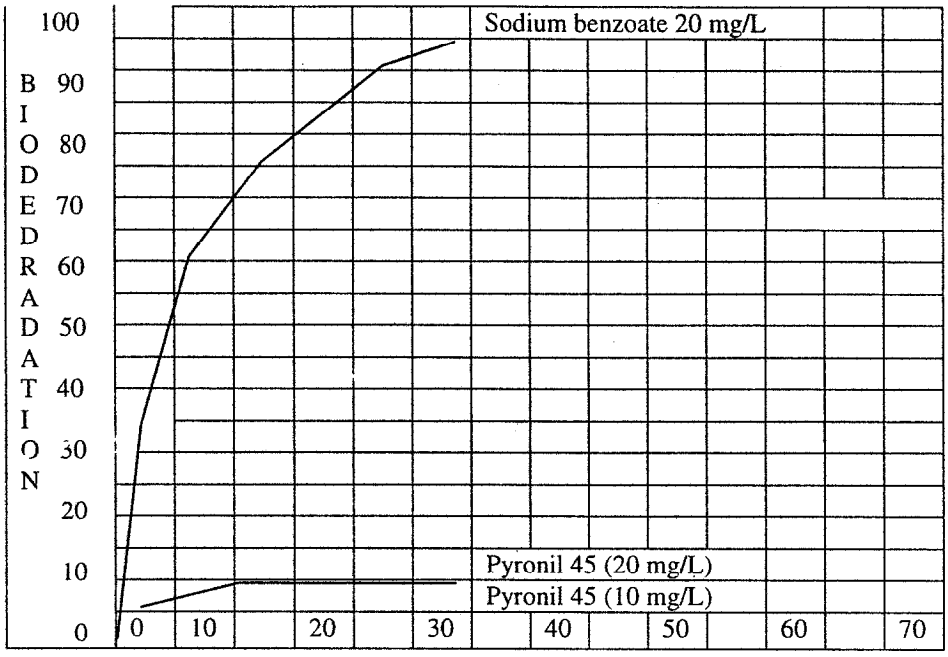
RESULTS: Determination of biodegradation: The biodegradation is calculated as the ratio of the biochemical oxygen demand (BOD) to the theoretical oxygen demand (ThOD). The ThOD of the test substance is 1.34 mg O_2/mg test substance. Oxygen consumption was only 0.06 and 0.05 mgO_2/mg, or 4% of its ThOD, at 2 and 10 mg/L after 10 days. These values indicate that the test material is not readily degradable. The degradation of sodium benzoate was not effected by the presence of the test material, demonstrating that it was not inhibitory to the bacterial inoculum.

The COD was 0.92 mgO2/mg, or 69% of its ThOD, demonstrating that the material was not completely oxidized.

CO2 production after 28 days was 1.1 and 2.3 mg for 10 and 20 mg/L test samples. This is only 2% of the TCO_2. These levels of TCO_2 after 28 days further demonstrate that the test material was not readily degradable. The DOC also illustrates that the test material was poorly soluble.
Temperatures of the test vessels ranged from 20.0 to 20.5°C, and the pH was 7.02-7.27 (at the start) and 6.42-6.99 (at the end).

The reference substance was 91% degraded after 28 days together with the cumulative CO_2 production in the control (20.1 mg) confirmed the viability of the inoculum, and validate the test results.

Figure1. The following figure portrays the biodegradation of the test material and sodium benzoate in 28 days.



DATA QUALITY: Study was conducted in accordance with a recognized scientific procedure for biodegradation and is based on OECD 301B and OECD 301D [Degradation-biotic degradation: Closed bottle test]. The study was conducted in accordance with GLP standards. The study provides sufficient information to support the conclusion that the test material is not readily biodegradable.

RELIABILITY:

1. w/o restriction [X]
2. w restriction [ ]
3. not reliable [ ]
4. not assignable [ ]

B. ACUTE TOXICITY TO FISH

1. REPORT NUMBER: AF-1

STUDY TYPE: Acute 96hour static toxicity to fish

TEST MATERIAL: Pale yellow clear liquid; Pyronil 45; Code 6605-57; halogenated phthalate ester; RC9927; FR-45B; purity 95%; specific gravity = 1.545 @25°C/4°C. Not readily water soluble. CAS No. 26040-51-7.

TESTING FACILITY: Life Science Research, Ltd

STUDY NUMBER(S): 89/PSV030/0194

SPONSOR: Pennwalt Corporation

REPORT ISSUED or STUDY COMPLETION DATE: June 30, 1989

TITLE OF REPORT: Pyronil 45: Acute Toxicity to Rainbow Trout.

RECOGNIZED METHOD: The test was carried out in accordance with OECD Test Guideline 203.

GLP: Yes

SPECIES: *Salmo gairdneri*, Rainbow Trout, obtained as fry and raised in the laboratory until they averaged 1.45 grams (mean wet weight).

WATER QUALITY and CONDITIONS DURING REARING: Hardness was 188-228 mg/l of $CaCO_3$; temperature was 13-14.3°C; pH was 7.3-7.8; dissolved oxygen concentration was 69-96%.

EXPOSURE PERIOD: 96 hours

ANALYTICAL MEASUREMENTS: Oxygen concentration, temperature and pH were measured during the test; as well as total hardness of the dilution water.

TEST DETAILS:
Preliminary toxicity and dispersion tests: Since the test material is not readily soluble, several preliminary tests were performed to determine the best methods for solubilizing the test material. Nominal concentrations were prepared. Ultrasound, vigorous shaking and acetone did not adequately produce homogenous dispersions based upon qualitative assessments (e.g. visual). Dimethyl formamide, triethylene glycol, methanol and ethanol were also tested. Based upon appearance (and not quantitative measurement) ethanol was selected to provide the best dispersion (e.g. least amount of undispersed test material in solution).

The test was performed as a static test, using 10 fish per each of the following concentrations: 0, 62.5, 125, 250, 500 and 1000 mg/L. Control fish were exposed to water alone or water plus ethanol (0.1 mg/L). Fish were observed for 45 minutes and again at 2 hours and 4 hours. Thereafter, fish were observed at 24, 48, 72 and 96 hours.

RESULTS:
Preliminary toxicity test: There were no mortalities. Analysis of samples performed during the preliminary toxicity test revealed that the test material had exceeded the nominal exposure levels, illustrating the test preparations were not homogenous. The temperature, pH, dissolved oxygen and hardness of the water were all measured.

Definitive toxicity test: There were no mortalities. In the definitive test, the analytical measurements of test preparations further illustrate the unreliability of the test concentrations. Test preparations were not homogenous. Undissolved material was observed in all dose vessels and in some cases was reported as "large globules". Once again water quality measurements were performed.

The LC50 (96 h) was not calculated because there were no deaths at the highest concentration of 1000 mg/L. However, the actual test concentration could not be verified by analytical measurements because the test substance was not dissolved and remained visible during the test. These test results cannot be interpreted.

Measurement of nominal and actual test concentrations:

Nominal Concentration mg/L	0 Hour measured values mg/L	Mean mg/L	96 Hour measured values mg/L	Mean mg/L	Overall mean mg/L
0	0, 0	0	0, 0	0	0
62.5	2.21, 5.16	3.7	2.68, 2.5	2.59	3.14
125	14.2, 5.6	9..9	2.45, 0,68	1.57	5.73
250	38.1, 23.3	30.7	0.5, 0.77	0.63	31.34
500	19.7, 37.5	28.6	2.6, 2.69	2.65	15.62
1000	41.1, 19.7	30.4	1.17, 0.67	0.92	15.66

DATA QUALITY: Study was conducted in accordance with a recognized scientific method for determining acute toxicity to fish. There was no mortality. The oxygen concentration was >90%. The insolubility of the test substance limits the quality and reliability of the study results.

RELIABILITY:

1. w/o restriction [ ]
2. w restriction [ ]
3. not reliable [X]
4. not assignable [ ]

The study is confounded by the insolubility of the test material resulting in poor analytical test concentrations. Based upon results obtained in the Daphnia study it is clear that the test material cannot be kept in solution at concentrations greater than 1 mg/L. The test results from the 96 hour fish toxicity study are not valid.

ACUTE TOXICITY TO AQUATIC INVERTEBRATES

1. REPORT NUMBER: AINV-1

STUDY TYPE: Acute static 48 hour Toxicity to Daphnia magna

TEST MATERIAL: Pale yellow clear liquid; Pyronil 45; Code 6605-57; halogenated phthalate ester; FR-45B; purity 95%; specific gravity = 1.545 @25°C/4°C. Not readily water soluble. CAS No. 26040-51-7.

TESTING FACILITY: Life Science Research, Ltd

STUDY NUMBER(S): 89/PSV031/0195

SPONSOR: Pennwalt Corporation

REPORT ISSUED or STUDY COMPLETION DATE: July 10, 1989

TITLE OF REPORT: Pyronil 45: Acute Toxicity to *Daphnia magna.*

RECOGNIZED METHOD: The test was carried out in accordance with OECD Test Guideline 202.

GLP: Yes

EXPOSURE PERIOD: 48 hours

SPECIES: Daphnia magna; obtained from National Institute for Applied Chemical Research in France. Cultures were maintained in tap water having a hardness of 200-250 mg/L $CaCO_3$, temperature of 20°C.

Cultures were fed 5 times a week with unicellular green alga (*Chlorella vulgaris*) and yeast. Gravid Daphnia were removed from culture, isolated and the juveniles produced were removed each day. Juveniles used in the test were 6-24 hours old.

EXPOSURE PERIOD: 48 hours

STATISTICAL METHODS: The EC50 was calculated using a binomial, moving average and/or probit analysis, using the number of Daphnia exposed and the number immobile and/or floating, at each nominal or measured concentration.

TEST DETAILS:

Preliminary tests: Several preliminary tests were conducted to determine the appropriate test conditions for solubilizing the test material, since it is poorly water soluble. In the first preliminary test, test concentrations were prepared ranging from 1 to 1000 mg/L. Water temperature was 19°C and hardness was 202-218 mg/l as $CaCO_3$. Each nominal concentration was prepared in water and shaken for 2 hours, then ultrasound for 15 minutes. At 1 and 10 m/l the test solutions were clear. At 100 and 1000 mg/l test material was visible as oily droplets on the surface. All daphnia were immobile after 48 hours, except at 1000 mg/l where all Daphnia were floating on the surface. Analysis of concentrations demonstrated that at 1 mg/L (nominal) actual concentrations were 0.84 – 0.87 m/l at the start and 0.72-0.75 mg/l at 48 hours. At 1000 mg/l (nominal) actual concentrations were 159-256 mg/l at the start and 141-203 mg/l at 48 hours.
In a second preliminary test, test material was prepared in acetone (10 mg/ml) and diluted in aqueous stock to provide nominal concentrations of 0.01, 0.1 and 1 mg/l. Water quality was the same as the first preliminary test and pH was 7.7-8.2. All Daphnia were immobile and floating on the surface after 48 hours at 1 mg/l; while at 0.01 and 0.1 mg/l they were mobile. All test solutions were clear. Two groups of 5 daphnia each were exposed at each concentration. Analytical measurements were not performed.

Definitive test: The deionized water used was produced from tap water in a water purification system. The test was carried out in a temperature controlled room with a light regime of 16 h of ambient light per day, provided by fluorescent tubes. The test was performed with *Daphnia magna* (water fleas) from a continuous culture maintained at the testing facility. The animals used in the test were less than 24 hours old at the beginning of the test and were obtained from parent animals having an age of 2-4 weeks.

The test was performed as a static test lasting 48 hours. There were four vessels at each exposure concentration and two control groups. Each contained 20 daphnia. The daphnia were exposed to the chosen concentrations of the test substance and immobility and sub-lethal effects were recorded at approximately 24 and 48 hours. The daphnia were considered immobile when they were not able to swim for 15 seconds after gentle agitation of the test vessel. In addition to immobility, sub-lethal effects such as floating at the surface were recorded.
The daphnia were randomly placed in the test solutions and the test vessels were positioned in a random manner. The test solutions were not aerated. The animals were not fed during the test. The nominal concentrations selected were 0.063, 0.125, 0.25, 0.5 and 1 mg/l.

ANALYTICAL MONITORING: Chemical analyses of the test concentrations revealed they were 77-88% of nominal at the start and 64-124% at 48 hours.

Nominal mg/l	Measured Pyronil 45 values (mg/l)			
	0 hours	mean	48 hours	mean
Control	0, 0	0	0, 0	0
0.063	0.071, 0.026	0.049	0.062, 0.093	0.078
0.125	0.119, 0.090	0.105	0.078, 0.133	0.106
0.25	0.218, 0.226	0.22	0.174, 0.158	0.166
0.5	0.393, 0.470	0.432	0.270, 0.366	0.318
1.0	0.730, 0.803	0.767	1.09, 0.707	0.899

RESULTS: The 48 hour EC50, based upon nominal concentrations, was 0.38 mg/l for immobile Daphnia, and 0.34 mg/l for combined immobile plus floating Daphnia. The 48 hour EC50, based upon actual concentrations, was 0.30 mg/l for immobile Daphnia, and 0.27 mg/l for combined floating plus immobile. The lowest concentration produced 5% immobile and 20% floating; whereas the highest concentration produced 95% immobile.

Nominal Pyronil 45 concentrations mg/l	Numbers pf Daphnia			
	Mobile		Immobile	
	Submerged	Floating	Submerged	Floating
24 hours				
Control	20	0	0	0
Acetone	20	0	0	0
0.063	20	0	0	0
0.125	19	1	0	0
0.25	12	7	0	1
0.5	4	14	0	2
1.0	0	7	0	13
48 hours				
Control	20	0	0	0
Acetone	20	0	0	0
0.063	16	3	0	1
0.125	20	0	0	0
0.25	16	1	0	3
0.5	3	1	0	16
1.0	0	1	1	18

Observation Times	EC50 nominal mg/l	EC50 measured mg/l
24 hour – immobile	0.84	0.68
24 hour – combined	0.29	0.24
48 hour – immobile	0.38	0.30
48 hour - combined	0.34	0.27

DATA QUALITY: This study was conducted in accordance with a recognized scientific procedure for determining acute toxicity to aquatic invertebrates. Special techniques had to be incorporated in order to insure that the test material was soluble; i.e. solubilize in acetone, ultra-sound, dilute concentrations.

RELIABILITY:

1. w/o restriction [ ]
2. w restriction [X]
3. not reliable [ ]
4. not assignable [ ]

NOTE: Several preliminary tests were performed in order to determine the best method to solubilize the test material. The definitive test used low concentrations and acetone in order to keep the test material in solution. Although test concentrations varied they were not considered significant enough to invalidate the study.